Complete and assembled genome sequence of an NDM-9- and CTX-M-15-producing Klebsiella pneumoniae ST147 wastewater isolate from Switzerland

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Abstract: OBJECTIVES: Carbapenem-resistant Klebsiella pneumoniae have emerged worldwide and represent a major threat to human health. Here we report the genome sequence of K. pneumoniae 002SK2, an NDM-9- and CTX-M-15-producing strain isolated from wastewater in Switzerland and belonging to the international high-risk clone sequence type 147 (ST147). METHODS: Whole-genome sequencing of K. pneumoniae 002SK2 was performed using Pacific Biosciences (PacBio) single-molecule, real-time (SMRT) technology RS2 reads (C4/P6 chemistry). De novo assembly was performed using Canu assembler, and sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). RESULTS: The genome of K. pneumoniae 002SK2 consists of a 5.4-Mbp chromosome containing blaSHV-11 and fosA6, a 159-kb IncFIB(K) plasmid carrying the heavy metal resistance genes ars and sil, and a 77-kb IncR plasmid containing blaCTX-M-15, blaNDM-9, blaOXA-9 and blaTEM-1. CONCLUSIONS: Multidrug-resistant K. pneumoniae harbouring blaNDM-9 and blaCTX-M-15 are spreading into the environment, most probably via wastewater from clinical settings.

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Complete and assembled genome sequence of an NDM-9 and CTX-M-15 producing *Klebsiella pneumoniae* sequence type 147 wastewater isolate from Switzerland

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Abstract

Objectives: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) have emerged worldwide and represent a major threat to human health. Here, we report the genome sequence of *K. pneumoniae* 002SK2, an NDM-9 and CTX-M-15 producing strain isolated from wastewater in Switzerland and belonging to the international high-risk clone ST147.

Methods: Whole genome sequencing of *K. pneumoniae* 002SK2 was performed using Pacific Biosciences (PacBio) single-molecule real-time (SMRT) technology RS2 reads (C4/P6 chemistry). *De novo* assembly was performed using canu assembler, and sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline.

Results: The genome of *K. pneumoniae* 002SK2 consists of a 5.4-Mbp chromosome containing $bla_{\text{SHV-11}}$ and *fosA6*, an IncFIB(K) 159-kb plasmid carrying heavy metal resistance genes *ars* and *sil*, and an IncR 77-kb plasmid containing $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-9}}$, $bla_{\text{OXA-9}}$ and $bla_{\text{TEM-1}}$.

Conclusions: Multidrug resistant *K. pneumoniae* harbouring $bla_{\text{NDM-9}}$ and $bla_{\text{CTX-M-15}}$ are spreading into the environment, most probably via wastewater from clinical settings.

Keywords

K. pneumoniae ST147, $bla_{\text{NDM-9}}$, $bla_{\text{CTX-M-15}}$, genome analysis

Klebsiella pneumoniae is not only ubiquitous in the environment and a common intestinal commensal, but also an important human pathogen, causing both nosocomial and community acquired infections. *K. pneumoniae* sequence type (ST)147 has emerged as a major international high-risk nosocomial clone and has recently been associated with VIM-1, NDM-1 and KPC-2 carbapenemases in many countries [1]. The carbapenemase NDM-9 differs from NDM-1 by a single amino acid substitution (E152K) and was first reported in the clinical *K. pneumoniae* ST107 isolate PPH1303 from China in 2013, followed by its detection in the *mcr-1* harbouring *Escherichia coli* strain THJS02 and in three environmental *K. variicola* isolates GJ1, GJ2, and GJ3 in South Korea [2].

K. pneumoniae isolate 002SK2 was isolated from wastewater in Basel, Switzerland in December 2015 [3]. DNA extraction was performed with the Wizard® Genomic DNAPurification Kit according to the manufacturers protocol (Promega AG, Dübendorf, Switzerland). The genome was sequenced at the Functional Genomics Center Zurich (FGCZ), Switzerland, using two single-molecule real-time (SMRT) cells on a PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA). The raw reads were filtered using the RS Filter Only protocol in the SMRT-portal (Pacific Biosciences) with 0.85 as polymerase read quality cut off and a minimal length of 500 bp. A total of 120,283 reads with an average length of 12,411 bp were selected, corresponding to 1,492,829,662 sequenced basepairs and a genome coverage of approximately 280 fold. The reads were assembled using the Canu assembler [4] with the option "-pacbio-raw" and an estimated genome size of 5.4 Mbp. The Canu output consisted of 4 contigs which were further polished in CLC workbench 7 (CLC, Aarhus, Denmark). The chromosomal origin of replication was identified using DoriC 5.0 and plasmid origins of replications were determined by PlasmidFinder 1.3. The genome was annotated by the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (GPAP) server.

The genome of *K. pneumoniae* 002SK2 has a G+C content of 57.4% and consists of a 5,393,461-bp chromosome with the origin of replication situated upstream of *gidA*, a common feature in *Klebsiella* species. The start of the sequence was set at 9 bp upstream of the first DnaA box in the origin region. Two extra-chromosomal elements of 159,714 and 77,809 bp, designated p002SK2-A and p002SK2-B, respectively, were identified. The PGAP server predicted a total of 5356 protein-coding sequences.

The chromosome of *K. pneumoniae* 002SK2 contains the ß-lactam resistance gene $bla_{\text{SHV-11}}$ and the fosfomycin resistance gene *fosA6*.

The 159,714-bp plasmid p002SK2-A (IncFIB(K)) carries no acquired antibiotic resistance genes but contains an *ars* operon encoding for arsenic resistance, *cop* genes encoding copper resistance, and *sil* system components mediating silver resistance. Plasmid 77,809-bp p002SK2-B (IncR) carries genes encoding resistance to aminoglycosides (*aacA4*, *aph(3')-VI*), ß-lactams ($bla_{\text{TEM-1}}$, $bla_{\text{OXA-9}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{NDM-9}}$), bleomycin (*ble*), fluoroquinolones (*qnrS*), and rifampin (*arr-2*), and the mercury resistance (*merc*) operon. The plasmid p002SK2-B does not harbour *tra* genes for conjugal transfer, but contains a toxin/antitoxin (A/T) *VapBC* operon.

The $bla_{\text{NDM-9}}$ gene is located in a region of ~ 7.5 kb bracketed by a 172 bp truncated insertion sequence (IS)*Aba14* (100% identity to GenBank accession number CP001921) and IS*26* (Figure 1). The $bla_{\text{NDM-9}}$ gene is located downstream of IS*Aba125*, representing a genetic arrangement highly conserved among NDM-producing Gram-negative species. IS*Spu_2* (GenBank accession number NC_009438) transposed into the IS*Aba125* element at bp position 877 of the IS*Aba125* transposase gene (Figure 1). An antimicrobial resistance gene conferring resistance to aminoglycosides, *aph(3')-VI* is located upstream of IS*Aba125* and downstream of the truncated ISAba14. Except for the IS*Spu_2* transposase region, the upstream and downstream environment of the $bla_{\text{NDM-9}}$ gene shares high similarity with that

of the *bla*$_{NDM-1}$ gene described in *K. pneumoniae* KP617 isolated in South Korea (GenBank accession number CP012754.1). It therefore appears that the mobilization events that were at the origin of the acquisition of the *bla*$_{NDM-9}$ gene in *K. pneumoniae* 002SK2 are different from those of the *bla*$_{NDM-9}$ genes in the nucleotide databases (GenBank accession numbers GJ1, CP017281-84; GJ2, CP017849-53; and GJ3, CP017285-89, respectively) [2].

Isolates like *K. pneumoniae* ST147 002SK2 highlight the dissemination of pathogenic MDR, NDM-9 producing strains into the environment. Careful monitoring of bacteria carrying carbapenemase genes in the environmental and clinical settings is warranted.

Sequence and annotation data of the *K. pneumoniae* 002SK2 genome have been deposited at GenBank under accession numbers CP025515 (chromosome), CP025516 (p002SK2-A), and CP025517 (p002SK2-B). This is the first version of this genome.

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Competing interests

None declared.

Ethical approval

Not required.

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Figure legend

Linear map of the *bla*$_{NDM-9}$ encoding region of p002SK2-B. Antimicrobial resistance genes are coloured in red, insertion sequences (IS) are shown in dark or light blue, other genes are shown in burgundy.

aph(3')-VI, gene coding for aminoglycoside 3'-phosphotransferase; *bla*$_{NDM-9}$, gene coding for the New Delhi metallo-ß-lactamase; *ble*, bleomycin resistance gene, *cutA*, gene coding for divalent-cation tolerance protein CutA: *sdbC,* gene coding for α-N-acetylgalactosaminidase; *trpf*, gene coding for N-(5'-phosphoribosyl)anthranilate isomerase.